CELL-CONTAINING CONTAINER AND METHOD FOR PRODUCING SAME

BACKGROUND OF THE INVENTION

Field of the Invention

[0001] The present invention relates to a cell-containing container and a method for producing the same. Priorities are claimed on Japanese Patent Application No. 2020-061251, filed on Mar. 30, 2020, and Japanese Patent Application No. 2021-010564, filed on Jan. 26, 2021, the content of which are incorporated herein by reference.

Description of Related Art

[0002] An action potential of nerve cells may be used to evaluate efficacy or toxicity with respect to the nerve cells. As one of methods for detecting and evaluating the action potential of the nerve cells, an evaluation method using a Microelectrode Array (MEA) is known. The MEA is an array of tiny electrodes placed on a substrate on which cells are cultured, and can detect an electrical activity of cells.

[0003] However, it is known that when the nerve cells derived from human iPS cells are cultured on the MEA and the action potential is detected, it is necessary to culture the nerve cells at higher density and for a longer period of time than in a case of using nerve cells derived from animals other than human.

SUMMARY OF THE INVENTION

[0004] The inventors have found that when iPS-derived nerve cells are cultured at high density for a long period of time, cells may aggregate and detach from a culture surface of a culture container.

[0005] For example, Patent Document 1 (Japanese Unexamined Patent Application, First Publication No. 2018-117567) describes a cell culture apparatus having an object of appropriately maintaining and stabilizing a culture environment for living cells. The cell culture apparatus includes a culture tank for culturing living cells, an online/in-line monitoring device, a cell state determination device having a sterile sampling device, an analyzer, a data collection device, a data analyzer, and a cluster analysis function, and an operation control compensator having a reference function of cell reaction model for each cluster or the like. However, Patent Document 1 does not describe that nerve cells aggregate in a case where the nerve cells are cultured at high density for a long period of time, and also not describes a specific parameter necessary for suppressing such aggregation of nerve cells.

[0006] An object of the present invention is to provide a technique for suppressing aggregation of nerve cells.

[0007] A cell-containing container according to the present invention includes: nerve cells; and a medium, in which the nerve cells adhere to a culture surface of the cell-containing container, an adhesion area between the nerve cells and the culture surface is 0.949 to 28 2 mm^2 per 80,000 nerve cells, and a concentration of glucose in the medium is 1 g/L or higher.

[0008] A method for producing a cell-containing container according to the present invention includes a step of incubating a container including nerve cells and a medium, under a culture condition, while replacing the medium at a predetermined timing, in which a concentration of glucose in the medium is maintained at 1 g/L or higher for a predetermined period.

[0009] According to the present invention, it is possible to provide a technique for suppressing aggregation of nerve cells.

BRIEF DESCRIPTION OF THE DRAWINGS

[0010] FIG. **1** is a graph showing results of measuring changes over time in glucose concentration in a nerve cell medium in Experimental Example 1.

[0011] FIGS. **2**A to **2**C show representative micrographs obtained by imaging nerve cells at 53rd day (D1V53) from seeding of nerve cells in Experimental Example 2.

[0012] FIG. **3**A shows a representative micrograph of nerve cells evaluated as Aggregation Level 1 in Experimental Example 3. FIG. **3**B shows a representative micrograph of nerve cells evaluated as Aggregation Level 2 in Experimental Example 3. FIG. **3**C shows a representative micrograph of nerve cells evaluated as Aggregation Level 3 in Experimental Example 3. FIG. **3**D shows a representative micrograph of nerve cells evaluated as Aggregation Level 4 in Experimental Example 3.

[0013] FIG. **4** is a graph showing changes over time in an aggregation level of nerve cells in Experimental Example 4.

[0014] FIG. **5** is a graph showing results obtained by examining a relationship between glucose concentration in a medium and an aggregation level of nerve cells in Experimental Example 5.

DETAILED DESCRIPTION OF THE INVENTION

[0015] [Cell-Containing Container]

[0016] The present invention provides a cell-containing container according to one embodiment including: nerve cells; and a medium, in which the nerve cells adhere to a culture surface of the cell-containing container, an adhesion area between the nerve cells and the culture surface is 0.5 mm^2 or more per 80,000 nerve cells, and a concentration of glucose in the medium is 1 g/L or higher.

[0017] As described below in Examples, the inventors have found that there is a relationship between aggregation of nerve cells and glucose concentration in a medium. In addition, it was clarified that in a case where nerve cells are cultured according to a normal protocol, the glucose concentration in the medium may be less than 1 g/L. Also, it was clarified that the aggregation of nerve cells can be suppressed by maintaining the glucose concentration in the medium at 1 g/L or more.

[0018] When the aggregation of nerve cells is suppressed, an action potential of nerve cells can be satisfactorily detected and evaluated using MEA or the like. Also, when the aggregation of nerve cells is suppressed, a state of nerve cells can be satisfactorily evaluated by other analysis means such as immunostaining.

[0019] The cell-containing container of the present embodiment is a culture of nerve cells in a culture container. The culture container may be a container generally used for cell culture, and a dish and a well plate are exemplary examples thereof. A diameter of the dish, the number of wells in the well plate, and the like can be appropriately selected according to an application.